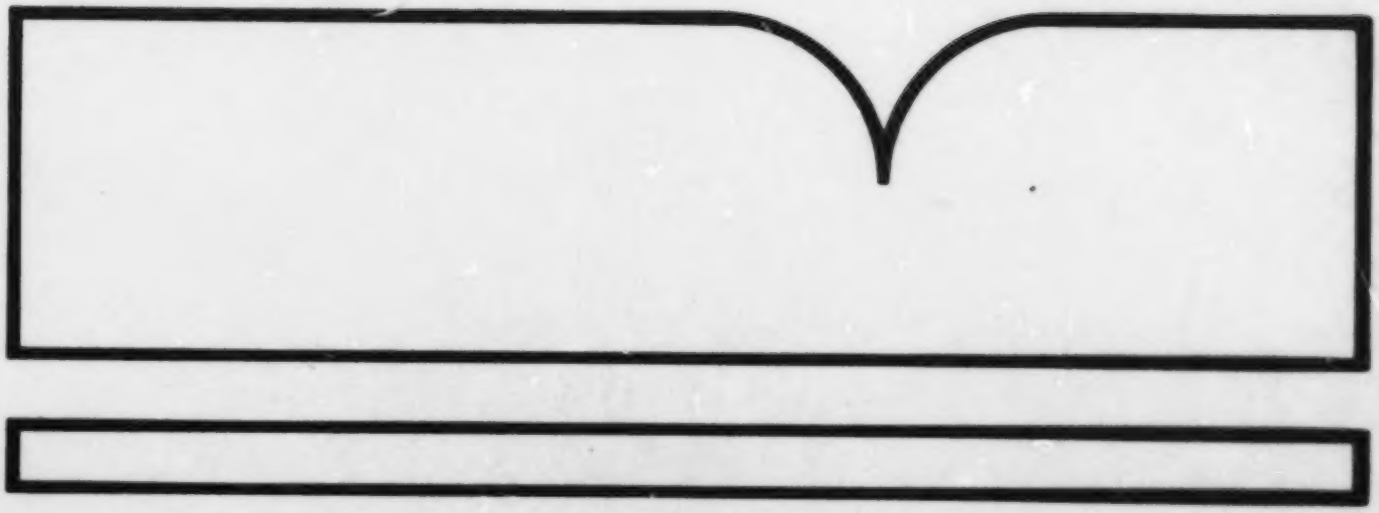
PB81-185696

'Crassostrea virginica' as an Indicator of Cadmium Pollution

(U.S.) Environmental Research Lab.
Narragansett, RI

Mar 81



U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3
EPA-600/J-80-236	journal article	PB81-185696

4. TITLE AND SUBTITLE	5. REPORT DATE
Crassostrea virginica as an indicator of cadmium pollution	March 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
G.E. Zaroogian	ERLN-J130
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U.S. Environmental Protection Agency Environmental Research Laboratory Narragansett, RI 02882	AARB1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Environmental Research Laboratory Narragansett, RI 02882	journal article
	14. SPONSORING AGENCY CODE EPA/600/05

15. SUPPLEMENTARY NOTES
REFERENCE: Marine Biology 58, 275-284, 1980

16. ABSTRACT

As much as 89,176 and 292 mg Cd kg^{-1} dry weights were accumulated by adult Crassostrea virginica after treatment for 40 wk with 5, 10 and 15 mg Cd kg^{-1}, respectively, in flowing seawater at ambient salinity and temperature without mortalities. Cadmium accumulation increased with increased concentrations of cadmium in seawater; greater amounts were accumulated during the summer months. At seawater temperature below 6°C cadmium concentrations in the total soft parts varied significantly between treatments, but not within treatments. Cadmium concentration in the total soft parts varied inversely with dry weight, whereas cadmium content varied directly.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release Unlimited	20. SECURITY CLASS (This page)	22. PRICE

EPA Form 2220-1 (9-73)

Marine Biology 58, 275–284 (1980)

MARINE BIOLOGY
by Springer-Verlag 1980

EPA-600/J-80-236
Journal Article

Crassostrea virginica as an Indicator of Cadmium Pollution

G. E. Zaroogian

Environmental Research Laboratory, US Environmental Protection Agency; South Ferry Road, Narragansett, RI 02882, USA

Abstract

As much as 89, 176 and 292 μg Cd g^{-1} dry weight were accumulated by adult *Crassostrea virginica* after treatment for 40 wk with 5, 10 and 15 μg Cd kg^{-1}, respectively, in flowing seawater at ambient salinity and temperature without mortalities. Cadmium accumulation increased with increased concentration of cadmium in seawater; greater amounts were accumulated during the summer months. Uptake patterns measured as cadmium content were similar among the total soft parts, gill, mantle and visceral mass. A continuous increase of cadmium concentration in the visceral mass was observed. This differed from the uptake patterns observed as cadmium concentration in gill, mantle and total soft parts. Although cadmium accumulation in the total soft parts and the tissues was curvilinear over the entire study period, significant linear relations between cadmium concentration and time indicated a general increasing trend. At seawater temperatures below 6 °C, when oysters were not actively feeding, cadmium concentrations in the total soft parts varied significantly between treatments, but not within treatments. In the tissues, the rate of uptake expressed as cadmium concentrations was visceral mass>gill>mantle. Cadmium concentration in the total soft parts varied inversely with dry weight, whereas cadmium content varied directly. During spawning, cadmium concentration in the total soft parts increased, whereas the content decreased. Cadmium concentration decreased in mantle and gill but increased in the visceral mass during spawning, whereas cadmium content decreased in all tissues. Regression analyses indicated that during spawning dry weight decreased at the same rate in gill and mantle, but they lost less weight and lost it more slowly than visceral mass. Also, during spawning, cadmium content decreased in mantle and gill at the same rate but more slowly than in the visceral mass. In mid-August, Cd concentration decreased despite the continuous addition of cadmium to the seawater; however, Cd content increased, suggesting that organism weight was responsible for fluctuations in cadmium concentrations.

Introduction

Estuarine waters are being increasingly burdened by metal pollution. If such pollution continues, large populations of shellfish may be damaged.

Since oysters accumulate many trace metals from seawater (Kurland *et al.*, 1960; Brooks and Rumsby 1965, 1967; Craig, 1967; Pringle *et al.*, 1968; Shuster and Pringle, 1969; Romeril, 1971; Eisler *et al.*, 1972; Ratkowsky *et al.*, 1974; Cunningham and Tripp, 1975b; Kerfoot and Jacobs, 1976; Zaroogian *et al.*, 1979) it appeared that they would be a good indicator of metal concentrations in their environment.

Previous studies of cadmium accumulation in oysters have examined natural populations whose history is unknown with respect to natural exposure to cadmium. Others were laboratory studies in which oysters were exposed to cadmium concentrations greater than 15 μg kg^{-1}. Concentrations of cadmium greater than 15 μg kg^{-1} are higher than those which oysters would encounter in chronically stressed natural environments (Kopfler and Mayer, 1969; Elderfield, 1971).

This study was designed to evaluate oysters as a potential indicator of cadmium pollution and to determine the accumulation of cadmium from low seawater concentrations by the total soft parts and selected tissues of oysters.

Materials and Methods

Adult oysters *Crassostrea virginica* obtained from Long Island Sound, USA in October 1973 were used as test organisms (height, 10.2 ± 1.2 cm). The general health condition of the individuals was established by histo-

0025-3162/80/0058/0275/

logical examination at time of harvest and at the start of cadmium addition to test troughs. At the time of harvest, a sample of 50 oysters was taken to determine whether a relationship existed between dry weight and cadmium concentration or content.

Oysters were acclimatized for one month in 3.75 m x 30 cm wide x 25 cm deep fiberglass troughs prior to testing. The troughs were supplied with flowing unfiltered Narragansett Bay, USA seawater (29 to 32 ‰ S) at the rate of 161 min^{-1}. Oysters were held on a false bottom made of polyethylene grids raised 2.5 cm off the bottom by PVC pipe. Since the incoming seawater contained ample food organisms, the oysters did not receive supplementary feeding during holding or treatment periods. The ambient seawater temperature was continuously recorded during the experiment.

Nine troughs (3 for each cadmium concentration) received a cadmium chloride solution ($CdCl_2 \cdot 2\ 1/2\ H_2O$) and 3 troughs served as controls. A seawater-cadmium solution was metered into the troughs to produce calculated concentrations of 5, 10 and 15 μg Cd kg^{-1} when mixed with incoming seawater (16 l min^{-1}).

Cadmium concentrations in the experimental troughs were determined weekly using methods described by Davey and Soper (1975). Concentrations ranged from 4.7 to 5.3 ($\bar{x}$ = 5.1), 9.3 to 10.6 ($\bar{x}$ = 9.9) and 14.2 to 14.8 ($\bar{x}$ = 14.4) μg Cd kg^{-1} for the 5, 10 and 15 μg kg^{-1} treatments, respectively. Seawater in the control troughs contained 0.1 to 0.2 μg Cd kg^{-1}. No mortalities were observed in the control or experimental troughs throughout the study.

Five oysters were removed from each trough and analyzed for cadmium prior to dosing. From November 1973 to April 1974, five oysters were removed and analyzed bi-weekly. From April 1974 to the end of August 1974, oysters were sampled weekly. Total soft parts of oysters treated with 5 and 15 μg Cd kg^{-1} were analyzed for cadmium. Mantle, gill, adductor muscle and the remaining soft parts (visceral mass) were dissected and analyzed separately for control oysters and oysters treated with 10 μg Cd kg^{-1}.

Oyster tissues were dissected, dried at 100 °C for 24 h and weighed prior to ashing at 425 °C for 18 h. Concentrated HNO_3 was added to ashed samples which were then dried at 100 °C for 24 h and re-ashed for 18 h. When necessary, ashing and treatment with HNO_3 were repeated until a clean white ash was obtained. The final ash was dissolved in 5% HNO_3 and analyzed for cadmium by atomic absorption spectrophotometry. Results were expressed in μg Cd g^{-1} (ppm) dry weight (concentration) and μg cadmium (content). The cadmium values reported for the total soft parts of control and 10 μg kg^{-1}-treated oysters were derived from the sum of the parts.

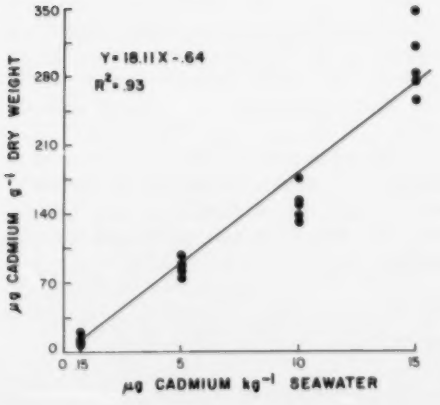


Fig. 1. ***Crassostrea virginica***. **Cadmium concentration in the total soft parts after 36 wk treatment, as a function of cadmium concentration in seawater**

Results

Accumulation

As much as 89, 176 and 292 μg Cd g^{-1} dry weight were accumulated by oysters from seawater containing 5, 10 and 15 μg Cd kg^{-1}, respectively.

Significant (P = .05) linear relations between time and cadmium concentration in the total soft parts existed in the 5, 10 and 15 μg Cd kg^{-1} treatments. The significance of the linear regressions indicated that the general trend of uptake as a function of time was increasing since positive slopes of 2.18, 3.81 and 7.54 were obtained for the 5, 10 and 15 μg Cd kg^{-1} treatments, respectively. The linear regressions also indicated that the rate of accumulation varied with the concentration of cadmium in the seawater. In an effort to further illustrate the relation of uptake and time, polynomial regressions of uptake onto time were investigated. Cadmium accumulation in the total soft parts of oysters treated with 5, 10 and 15 μg Cd kg^{-1} was best fit with a fourth degree polynomial regression model (all equations were significant at P = .05). The R^2 values for a fourth degree polynomial regression model of uptake means and time of exposure for the 5, 10 and 15 μg Cd kg^{-1} treatments were .79, .80 and .84, respectively; whereas the R^2 values for a linear regression model were .61, .64 and .71, respectively.

A highly significant linear relationship existed between cadmium concentration in the total soft parts and cadmium concentration in seawater (Fig. 1). Graphically, the line describing tissue cadmium concentration as a function of cadmium concentration in seawater (Fig. 1) passed through the origin. Thus, it appears that *Crassostrea virginica* does not regulate cadmium in its tissues. Also, a significant (P = 1.05) inverse relationship was observed between cadmium concentration in the total soft parts and dry weight (Fig. 2). However, a significant direct relationship existed between cadmium content in the total soft parts and dry weight (Fig. 3).

To determine whether the cadmium residues in the total soft parts were significantly different between

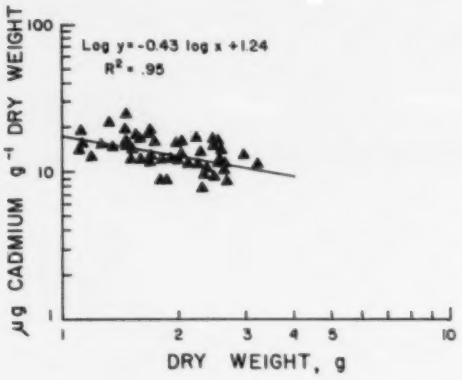


Fig. 2. *Crassostrea virginica*. Cadmium concentration in the total soft parts as a function of dry weight in a sample of 50 oysters taken at the same time that oysters were collected for the uptake study

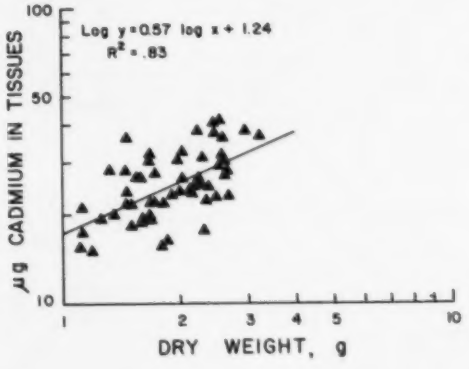


Fig. 3. *Crassostrea virginica*. Cadmium content (µg) in the total soft parts as a function of dry weight in a sample of 50 oysters taken at the same time that oysters were collected for the uptake study

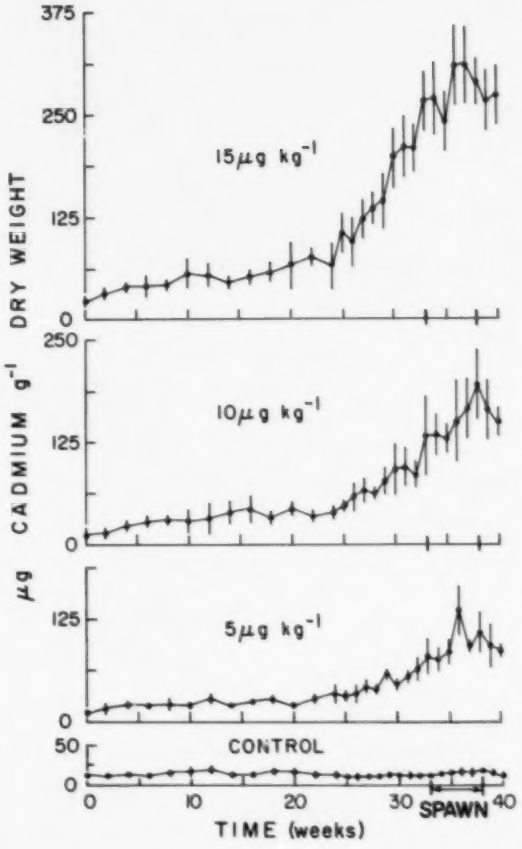


Fig. 4. *Crassostrea virginica*. Cadmium concentrations in the total soft parts after treatment of oysters with seawater containing ambient (control), 5, 10 and 15 µg Cd kg^{-1} for 40 wk (November 1973 through August 1974). Each symbol represents the mean cadmium concentration for each sample of 5 oysters ± 1 standard deviation

treatments, the least significant difference (LSD) was determined and compared to mean differences. The calculated $LSD_{.01}$ was 19.06; the differences between the means were $\bar{X}_5 - \bar{X}_0 = 35.0$, $\bar{X}_{10} - \bar{X}_5 = 27.4$ and $\bar{X}_{15} - \bar{X}_{10} = 63.5$.

Seasonal variations in patterns of uptake as tissue cadmium concentration were apparent when oysters were treated with ambient, 5, 10 and 15 µg Cd kg^{-1} (Fig. 4). Uptake patterns of tissue cadmium content reflected seasonal influences similar to those expressed as tissue concentrations, with the exception of the last several weeks of treatment (Fig. 5).

Uptake patterns expressed as cadmium content suggested that cadmium accumulation had not maximized and that cadmium content would continue to increase after 40 wk (Fig. 5). Figures 5, 6, 7, 8 display similarities in patterns of uptake expressed as cadmium content in the total soft parts, gill, mantle, and visceral mass.

In contrast to patterns of uptake expressed as cadmium content, patterns of uptake as cadmium concentration in the total soft tissue suggested that cadmium accumulation had maximized by mid-August (Week 38), with a subsequent loss of cadmium (Fig. 4). Uptake patterns as cadmium concentration in the gill, mantle and visceral mass were dissimilar to that in the total soft tissue (Figs. 4, 6, 7, 8). The uptake pattern of visceral mass more closely resembled that of the total soft tissue than that of the mantle or gill (Figs. 4, 6, 7, 8). Cadmium concentration increased continuously for 38 wk in both the visceral mass and total soft parts; however, a continuous decline was observed in the total soft parts, but not in the visceral mass (Figs. 4, 8) for the ensuing 2 wk. The uptake pattern as cadmium concentration in the visceral mass differed from those in the gill and mantle. A continuous increase in cadmium concentration occurred over the entire study period in

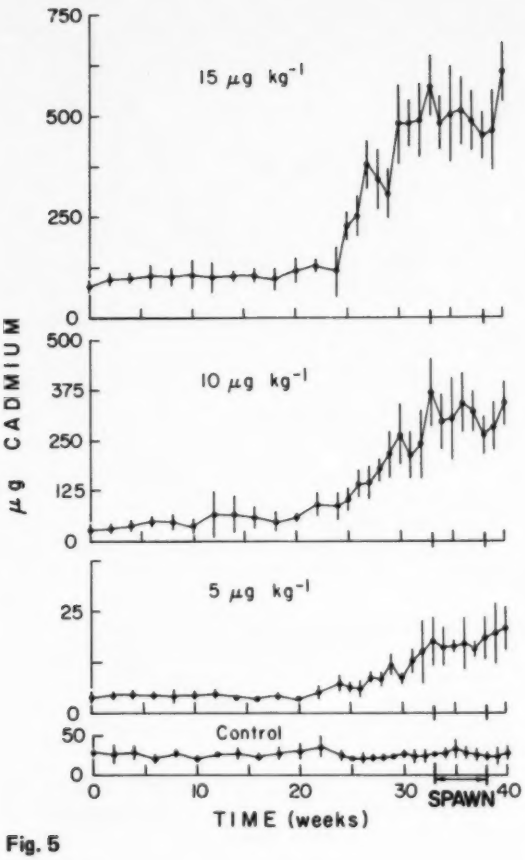


Fig. 5

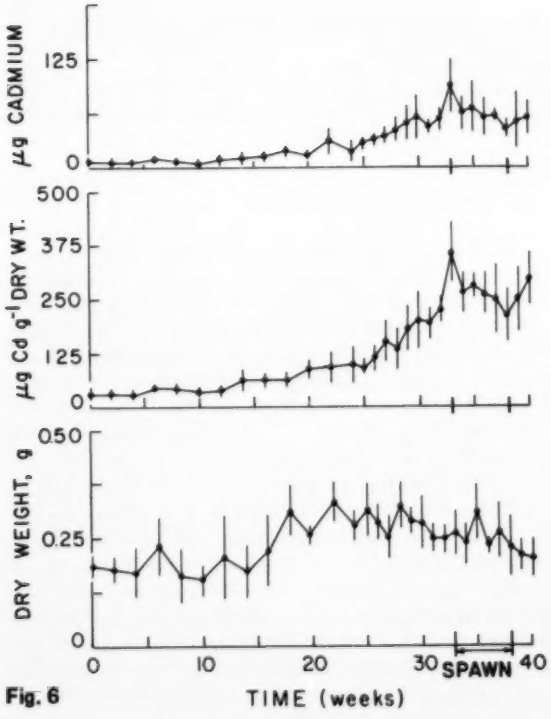


Fig. 6

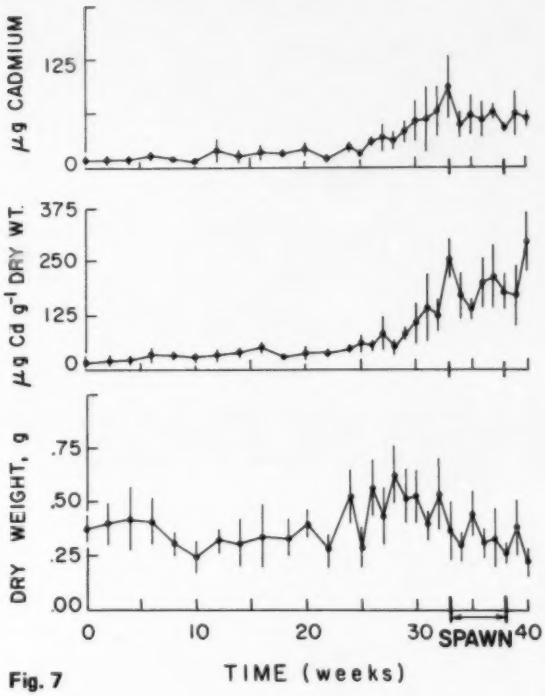


Fig. 7

Fig. 5. *Crassostrea virginica*. Cadmium content (μg) in the total soft parts after treatment of oysters with seawater containing ambient (control), 5, 10 and 15 μg Cd kg^{-1} for 40 wk (November 1973 through August 1974). Each symbol represents the mean cadmium content for each sample of 5 oysters ± 1 standard deviation

Fig. 6. *Crassostrea virginica*. Gill cadmium content, cadmium concentration and dry weight in oysters treated with 10 μg Cd kg^{-1} seawater for 40 wk (November 1973 through August 1974). Each symbol represents the mean value in the gill from each sample of 5 oysters ± 1 standard deviation

Fig. 7. *Crassostrea virginica*. Mantle cadmium content, cadmium concentration and dry weight in oysters treated with 10 μg Cd kg^{-1} seawater for 40 wk (November 1973 through August 1974). Each symbol represents the mean value in the mantle for each sample of 5 oysters ± 1 standard deviation

the visceral mass, but not in the gill and mantle (Figs. 6, 7, 8). A pattern similar to that in gill was observed in mantle tissue, in which a maximum cadmium concentration was reached by mid-July (33 wk), followed by a loss with a subsequent increase (Fig. 6, 7).

Cadmium accumulation was more rapid and tissue concentrations were higher in the gill than in the mantle or visceral mass during the first 33 wk of uptake (Figs. 6, 7, 8). Linear regression analyses indicated that each tissue accumulated cadmium at a rate which was significantly different from any other tissue at $P = .01$.

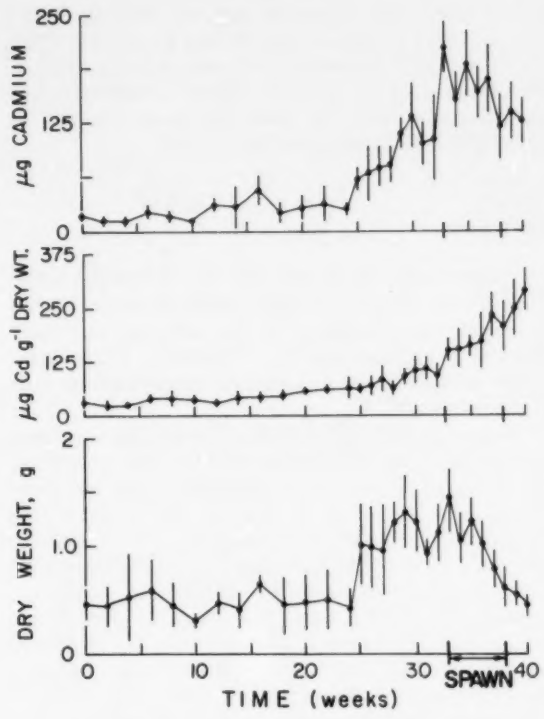


Fig. 8. *Crassostrea virginica*. Visceral mass cadmium content, cadmium concentration and dry weight in oysters treated with 10 µg Cd kg^{-1} seawater for 40 wk (November 1973 through August 1974). Each symbol represents the mean value in the visceral mass from each sample of 5 oysters ± 1 standard deviation

Tissue concentrations of cadmium were found to be greatest in gill > visceral mass ≥ mantle. Cadmium content was greatest in the visceral mass > gill ≥ mantle.

Regression analyses indicated that cadmium uptake expressed as concentration in the gill, mantle and visceral mass was curvilinear as a function of time.

Temperature

Linear regression analyses indicated that the effects of seawater temperature and cadmium concentration on uptake were highly related. The correlation coefficients were 0.51 and 0.58, respectively.

To determine whether the cadmium accumulation which occurred during the colder months was significant, separate regression analyses were performed on the data collected at temperatures below and above 6 °C. Tissue analyses of oysters treated at temperatures less than 6 °C indicated that small amounts of cadmium had been accumulated in the total soft tissues, but the increases were not significant.

The least significant difference was calculated to determine whether a significant difference in cadmium accumulation existed between treatments at temperatures below 6 °C. The $LSD_{.01}$ was 5.61 between treatments for that sampling period. The differences between the means were $\bar{X}_5 - \bar{X}_0 = 8.03$, $\bar{X}_{10} - \bar{X}_5 = 9.39$ and $\bar{X}_{15} - \bar{X}_{10} = 15.58$. Although cadmium accumulation within treatments was non-significant at temperatures below 6 °C, cadmium concentrations in the total soft tissues were significantly different between treatments. Greater variability existed within a sample than between sample means for a given treatment at seawater temperatures below 6 °C.

Significantly greater amounts of Cd were accumulated during Weeks 20 through 40 when the water temperature was above 6 °C. Regression analyses of cadmium concentration in the total soft parts onto time of treatment for study Weeks 20 to 40, when seawater temperature was above 6 °C, indicated that the data from the 5, 10 and 15 µg Cd kg^{-1} treatments were best fit by a fourth degree polynomial regression model. Regression models for cadmium concentration in the total soft parts onto time for all treatments (Weeks 20 to 40) were not significantly different from those obtained with the data from the entire study. The pattern of uptake as concentration at temperatures above 6 °C for gill and mantle remained curvilinear and was best fit with a fourth degree polynomial regression model. Uptake in the visceral mass was also curvilinear, but was best fit by a quadratic regression model. Though uptake was best fit by curvilinear functions, significant linear regression models of cadmium concentration in the total soft parts (5, 10 and 15 µg Cd kg^{-1} treatments) and each tissue onto time indicated a general trend of increase in body burden of cadmium at seawater temperatures above 6 °C.

Weight

A decrease in weight of total soft tissue of control oysters was observed during the winter months (Fig. 9). Late in February, weight began to increase with subsequent rapid growth in March and April, followed by a period of stabilization from May to mid-July (Fig. 9). Weight decreased during July and early August, but began to increase again in September (Fig. 9).

There is an indication of an inverse relationship between weight and tissue cadmium concentration during the first 24 wk (Figs. 4,9). This relationship is questionable, however, since it was established that no significant difference occurred between successive samples within a treatment during the first 20 wk of study.

During Weeks 25 to 35, fluctuations in weight were minimal and tissue cadmium concentrations increased significantly (Figs. 4, 9). After Week 35, there was an inverse relationship of weight and tissue concentration that appeared to influence the uptake patterns. It was during this period that the highest tissue cadmium concentration was observed (Fig. 4).

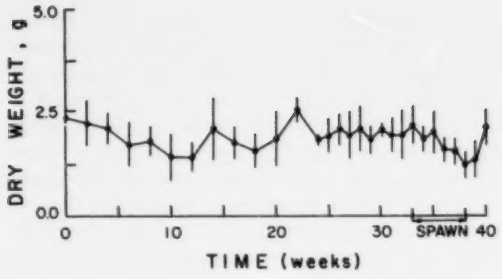


Fig. 9. *Crassostrea virginica*. Dry weight of the total soft parts as a function of time, from November 1973 through August 1974

Spawning

Spawning occurred in the experimental troughs during Weeks 33 to 38 of the study. During this period, cadmium concentration in treated oysters continued to increase although no change in cadmium content was apparent in the total soft parts (Fig. 5). Control data indicate that cadmium content decreased, as did weight, during spawning, whereas cadmium concentration continued to increase (Fig. 4, 5, 9).

Cadmium concentrations decreased in the gill and mantle tissue during spawning, but a continuous increase was observed in the visceral mass (Figs. 6, 7, 8). Cadmium content decreased in the gill, mantle and visceral mass in a pattern similar to that observed in the total soft parts (Figs. 5, 6, 7, 8).

Immediately after oysters had spawned, the cadmium concentrations in gill and mantle tissues increased dramatically while concentrations in the total soft parts decreased (Figs. 4, 6, 7). Also, cadmium content in the gill, mantle and visceral mass did not increase appreciably although that in the total soft parts did (Figs. 4, 6, 7, 8). The increase in cadmium content of the total soft parts appeared to be real because it occurred within all treatments (Fig. 5).

It was apparent from the linear regressions of tissue dry weight as a function of time during the spawning period, that gill and mantle had lines of the same slope and intercept, which were different from those of the visceral mass. Regression analyses indicated that gill and mantle lost weight at the same rate, but lost less weight and lost it more slowly than did the visceral mass. Regression analyses of cadmium content as a function of time during spawning established that gill, mantle and visceral mass had lines of the same slope, but that visceral mass had an intercept higher than that of gill and mantle. Thus, it appears that cadmium content decreased in gill, mantle and visceral mass at the same rate, but that less cadmium was lost from the gill and mantle than from the visceral mass.

The average weight loss observed in gill and mantle tissues of treated oysters during spawning was .04 and .09 g, respectively and was .82 g in the visceral mass. The average loss in cadmium content was 89.7 µg in the visceral mass, 46.4 µg in the gill and 45.7 µg in the mantle. Loss of weight in the gill and mantle was small (*ca* 15 and 25% respectively) in comparison to that of the visceral mass (*ca* 57%), yet gill and mantle each lost approximately 50% of their cadmium content as opposed to a 42% loss in the visceral mass.

Discussion

Regression analyses of the data for the study corroborated our observation that cadmium accumulation by oysters was curvilinear. It also indicated that more variability in uptake can be explained by a curvilinear model at high seawater cadmium concentrations than by a linear model.

Shuster and Pringle (1969) reported that cadmium accumulation by *Crassostrea virginica* was curvilinear and occurred in 3 phases when temperature was maintained at 20 °C for 20 wk. Kerfoot and Jacobs (1976) suggested that cadmium accumulation by *C. virginica* was linear after 67 d. Zaroogian *et al.* (1979) reported that lead accumulation by *C. virginica* was curvilinear, perhaps due to the influence of spawning. Cunningham and Tripp (1973) found mercury uptake in *C. virginica* to be curvilinear and also implicated spawning as a factor. However, Cunningham and Tripp (1975b) established that mercury uptake in *C. virginica* was also curvilinear when spawning was not a factor.

Galtsoff (1964), Pringle *et al.* (1968) and Roosenberg (1969) reported seasonal differences in metal concentrations in oysters, with lesser concentrations observed in the fall and winter. Frazier (1975) analysed oysters which had been held in suspended trays in Rhode River, USA for 21 months and reported that seasonal fluctuations in cadmium concentrations occurred in early August, followed by a gradual decrease in concentration to October.

In our study, cadmium accumulation followed a seasonal pattern similar to that reported by Frazier (1975). We found that the greatest cadmium accumulation started in April when mean seawater temperature was 6° to 9 °C and continued to mid-August, after which tissue concentration declined.

Phillips (1976) suggested that seasonal fluctuations of cadmium in mussels *Mytilus edulis* were due in part to seasonal variations in wet weight. It appeared that mussels with seasonally high wet weights had seasonally low concentrations of trace metals. It was suggested that total metal content remained almost constant throughout the year, but that the mussels' weight change caused fluctuations in metal concentrations in the soft tissues.

The decline in cadmium residues we observed in this study could be attributed to variations in weight. We found lower concentrations of cadmium in oysters with high dry weights than in those with low weights, despite a higher cadmium content (µg) in larger individuals. We also observed fluctuations in cadmium content of control oysters which appeared to be closely correlated with weight changes. Other authors have reported similar

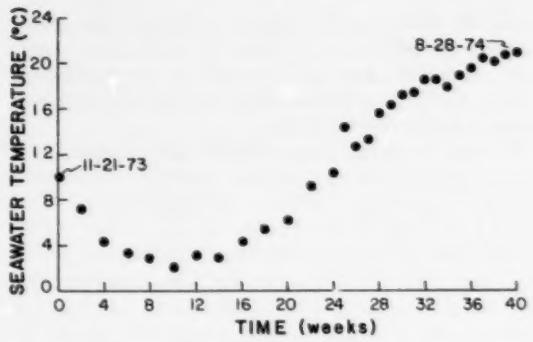


Fig. 10. Weekly mean seawater temperature during cadmium treatment

findings (Ayling, 1974; Cunningham and Tripp, 1975a; MacKay *et al.*, 1975; Watling and Watling, 1976).

Cunningham and Tripp (1975a) suggested that the more rapid growth rate in small oysters would account for more rapid metal incorporation. MacKay *et al.* (1975) proposed that the growth rate of oysters was greater than the rate of metal accumulation, but that growth still influenced increased uptake. In both instances, an increased metabolic activity in small oysters is implicated in producing higher cadmium concentrations in smaller oysters.

Binding by cellular constituents whose concentrations are directly related to metabolic activity could also account for higher cadmium concentrations found in smaller oysters. Read (1962) and Bayne (1971) reported that small *Mytilus edulis* have a higher metabolic activity than larger individuals. Casterline and Yip (1975) found that significant amounts of non-dialyzable cadmium in oysters were associated both with a large molecular weight protein fraction and with a soluble fraction which tested negative for proteins. Cadmium in mussels was bound principally to low molecular weight proteins which were synthesized in response to the cadmium uptake (Noel-Lambot, 1976). Chow *et al.* (1978) found that approximately 23% of the cadmium in oyster homogenate was present as bound cadmium.

Bryan (1973) noticed seasonal variations in metal concentration in *Pecten maximus* and *Chlamys opercularis*, with the highest metal concentrations in tissues at times when phytoplankton productivity was low. He suggested that although the heavy metal concentration in seawater influences accumulation of heavy metals by bivalves, phytoplankton productivity may be more important in bioaccumulation of metals. Like Bryan (1973), we also observed lower concentrations and higher contents of cadmium during phytoplankton blooms. However, concurrent with the blooms were weight increases in the oysters which we believe were responsible for the fluctuations in cadmium concentrations and contents.

Cadmium accumulation in our studies was much more variable among the oysters exposed to 10 and 15 $\mu g\ kg^{-1}$ than those exposed to 5 $\mu g\ kg^{-1}$. The cause for the increased variability at higher concentrations is unknown. Regardless of the cause, increased variability in uptake at higher concentrations is an indication of the need to study metals like cadmium in concentrations which correspond closely to those which might occur in impacted waters.

Galtsoff (1964) reported that reduced water flow through gills and cessation of feeding in oysters occurred at water temperatures below 6 °C. The lowest seawater temperature observed during this study (2 °C) occurred at 10 weeks (Fig. 10). A measured decrease in cadmium concentration and content in gill and mantle tissues corresponded with low temperature. The reduction in tissue cadmium concentrations and content that we observed at temperatures less than 6 °C was probably due to reduced pumping activity of the oyster (i.e., reduced exposure).

The significant differences in cadmium concentrations that we observed between treatments at temperatures below 6 °C indicated that *Crassostrea virginica* probably accumulated Cd directly from estuarine waters, independent of food resources. Frazier (1975) reported that cadmium concentration in oyster tissues gradually increased throughout the winter months at water temperatures below 10 °C, when little feeding occurred. In this study, slight increases in cadmium accumulation within treatments were observed at temperatures below 6 °C; however, with the exception of cadmium concentration in gill tissues, these increases were not significant.

After 40 wk of accumulation, uptake patterns expressed as cadmium concentrations in gill, mantle and visceral mass were very similar. However, prior to spawning (33 wk), the pattern of cadmium distribution per gram of tissue was: gill>mantle>visceral mass. Based on Cunningham and Tripp's (1975b) summary of the literature about the distribution of heavy metals in tissues of bivalve molluscs, it is evident that gills contain consistently higher concentrations of metals than other tissue. Pringle *et al.* (1968) proposed that the gill's large surface area and the gill mucous, which may act in ion exchange, contribute to high metal concentrations found in gill tissues. Brooks and Rumsby (1967) also observed relatively large concentrations of cadmium in the gills and suggested that adsorption of cadmium ions to the mucous sheets of the gill contributed to the high concentrations found there.

Galtsoff (1964) reported that gills serve as an excretory organ. Cadmium could, therefore, be eliminated through the gills. If gills are functioning in both uptake and excretion of cadmium, that could account for localization of cadmium in the gills.

Contrary to the literature, our cadmium content data indicated that cadmium distribution in the tissues was: visceral mass>gill≥mantle. Since the visceral mass constituted the largest mass of the tissues studied, we probably observed an effect of weight on tissue cadmium content.

During spawning the average weight of the total soft parts decreased by 51%. Concurrent with the weight

loss was a 27% decrease in the average cadmium content in the total soft parts. The weight of the visceral mass comprises 52% of the average weight of the total soft parts. Fifty-eight percent of the average cadmium content of the total soft parts was found in the visceral mass. During spawning, 57% of the average weight and 42% of the average cadmium content in the visceral mass was lost. From this, we calculate that 58% of the average weight lost and 89% of the average cadmium content lost from total soft parts during spawning can be attributed to the loss of weight and cadmium content in the visceral mass. This further supports arguments that increased cadmium concentrations in the total soft parts and visceral mass during spawning are due primarily to a proportionally greater loss in weight than in cadmium content.

More dramatic differences between the percentage losses of weight and cadmium content during spawning might be anticipated in the visceral mass if gonads are assumed to be primarily responsible for cadmium fluctuations. However, Grieg *et al.* (1975) suggested that the amount of metal transferred from adult to egg is fairly constant and not dependent on the amount of metal in the adult oyster. Other organs included in the visceral mass, such as the heart and kidney, may play equally important roles in affecting visceral mass cadmium concentrations. Brooks and Rumsby (1967) observed relatively large concentrations of cadmium in oyster hearts. In *Mytilus edulis*, of the tissues Schulz-Baldes (1974) studied, the kidney showed the highest rate of lead uptake.

As indicated by the uptake patterns in total soft parts of control oysters in this study, weight fluctuations could be responsible for fluctuations in tissue cadmium concentrations at seawater cadmium concentrations of 1 $\mu g\ kg^{-1}$ or less. However, uptake was continuous at seawater cadmium concentrations of 5 $\mu g\ kg^{-1}$ or more, except during spawning when a loss of tissue cadmium content was observed despite the continuous addition of cadmium. The changes in uptake by individual tissues we studied appeared to follow the changes in weight more closely than that of the total soft parts for all treatments.

Frazier (1975) reported that cadmium concentration and content were at a maximum while weight was decreasing. This is contrary to our findings which indicated a direct correlation between cadmium content and dry weight. In addition, the patterns of cadmium uptake by *Crassostrea virginica* that Frazier (1975) reported were very similar for content and concentration. This is also contrary to our findings, since weight decreases were reflected as increases in cadmium concentration in our study.

Windom and Smith (1972) suggested that the speciation of the metal is an important consideration in metals uptake by *Crassostrea virginica*. When organic mercury was substituted for mercuric chloride, Cunningham and Tripp (1975a) observed a four-fold increase in mercury accumulation by *C. virginica*. George and Coombs (1977) reported that the chemical form of cadmium affects the rate of cadmium uptake in *Mytilus edulis*, in that the complexed or chelated cadmium produced a doubling of final concentration in all tissues. In our study, cadmium was not detected in the particulate fraction and the cadmium added to the seawater was recovered in the soluble fraction.

Changes in metal concentration and content with weight fluctuations in certain shellfish have been reported (Ayling, 1974; Schulz-Baldes, 1974; Cunningham and Tripp, 1975a; Mackay *et al.*, 1975; Phillips, 1976; Watling and Watling, 1976; Boyden, 1977; Zaroogian, 1979). It is possible that computations using only weight to normalize uptake data has lead to erroneous conclusions. Because metabolic activity is strongly implicated in metal uptake (Cunningham and Tripp, 1975a; Mackay *et al.*, 1975; Boyden, 1977), it appears desirable to use the activity rates of a conservative cellular constituent such as nitrogen (protein), which is associated more directly with metabolic activity of a given tissue, to normalize data.

Butler *et al.* (1971) recommended that a biological indicator should satisfy the following requirements: a sedentary or sessile existence, abundance in the study region, long lived and easy to sample, be of reasonable size to insure adequate tissue for analysis, and must be able to accumulate the pollutant without being killed by the concentrations being monitored. Haug *et al.* (1974) suggested that, in addition, the organism should tolerate brackish water and accumulate toxicants with a high concentration factor and that a simple correlation exist between metal content of the organism and average metal content in surrounding waters. We feel that it is equally as important that the organism does not regulate its uptake of the metal being monitored. We feel that *Crassostrea virginica* satisfies these requirements for cadmium. In addition, the fact that cadmium is not depurated by *C. virginica* (Greig and Wenzloff, 1978; Zaroogian, 1979) enhances its usefulness as an indicator organism for cadmium pollution.

Our study indicates that spawning must be taken into account when using molluscs as indicator organisms. If oysters or other bivalve molluscs are to be used as indicators of metal pollution, proper determinations of metal concentration can only be derived from samples taken continuously over extended times, including both pre- and post-spawn samples.

Conclusions

Crassostrea virginica is an excellent bio-indicator of cadmium since accumulation was observed in the total soft parts and selected tissues at a seawater cadmium concentration of 5 $\mu g\ kg^{-1}$.

At seawater concentrations of .1–.2 μg Cd kg^{-1}, steady state concentrations of $14.1 \pm 4.2\ \mu g\ g^{-1}$ dry weight are attained in the total soft parts of oysters.

Crassostrea virginica is not a good indicator of cadmium pollution at seawater temperatures below 6 °C.

Weight decreases in spawning appear to result in increases in tissue cadmium concentrations.

Cadmium accumulation by *Crassostrea virginica* is highly related to seawater temperature and cadmium concentration.

Size is an important consideration when sampling oysters from natural populations since small oysters appear to have higher concentrations of cadmium than large oysters.

With the exception of cadmium concentration in the visceral mass, accumulation patterns in the tissues were similar to those of the total soft parts. Therefore, there appears to be no advantage in using tissues in lieu of total soft parts when studying cadmium accumulation in oysters.

A loss of weight disproportional to the loss of cadmium content in the visceral mass appears to account for the continuous increase of cadmium concentration in both the visceral mass and the total soft parts during spawning.

Acknowledgements. I am grateful to Messrs. W. Galloway and J. Sewall for their skilled technical assistance. Thanks are due to Dr. E. Davey and Mr. A. Soper for the seawater analyses and to Mr. R. Hennekey for critical review of the manuscript.

Literature Cited

Ayling, G. M.: Uptake of cadmium, zinc, copper, lead and chromium in the Pacific oyster *Crassostrea gigas* grown in the Tamar River, Tasmania. Water Research *8*, 729–739 (1974)

Bayne, B. L.: Oxygen consumption by three species of lamellibranch molluscs in declining ambient oxygen tension. Comp. Biochem. Physiol. *41A*, 955–970 (1971)

Boyden, C. R.: Effect of size upon metal content of shellfish. J. mar. biol. Assoc. U.K. *57*, 675–715 (1977)

Brooks, R. R. and M. G. Rumsby: The bioeochemstry of trace element uptake by some New Zealand bivalves. Limnol. Oceanogr. *10*, 521–527 (1965)

Brooks, R. R. and M. G. Rumsby: Studies on the uptake of cadmium by the oyster *Ostrea sinuata* (Lamarck). Aust. J. Mar. Freshwater Res. *18*, 53–61 (1967)

Bryan, G. W: The occurrence and seasonal variation of trace metals in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.). J. mar. biol. Assoc. U.K. *53*, 145–166 (1973)

Butler, P. A., L. Andreim, G. J. Bonde, A. Jernelov and D. J. Reisch: Monitoring organisms. F.A.O. *Fisheries Reports*, No. 99, supplement 1, 101–112 (1971)

Casterline, J. L., Jr. and G. Yip: The distribution and binding in oyster, soybean and rat liver and kidney. Arch. of Envir. Contam. Toxicol. *3*, 319–329 (1975)

Chow, C. L., J. F. Uthe and E. G. Zook: Polaragraphic studies on the nature of cadmium in scallop, oyster and lobster. J. Fish. Res. Bd Can. *35*, 409–413 (1978)

Craig, S.: Toxic ions in bivalves. J. Am. Osteop. Assoc. *66*(9), 1000–1002 (1967)

Cunningham, P. A. and M. R. Tripp: Accumulation and depuration of mercury in the American oyster *Crassostrea virginica*. Mar. Biol. *20*, 14–19 (1973)

Cunningham, P. A. and M. R. Tripp: Factors affecting the accumulation and removal of mercury from tissues of the American oyster *Crassostrea virginica*. Mar. Biol. *31*, 311–319 (1975a)

Cunningham, P. A. and M. R. Tripp: Accumulation, tissue distribution and elimination of $^{203}Hg\ Cl_2$ and $CH_3\ ^{203}HgCl$ in the tissues of the American oyster *Crassostrea virginica*. Mar. Biol. *31*, 321–334 (1975)

Davey, E. W. and A. E. Soper: Apparatus for the sampling and concentration of trace metals from seawater. In analytical methods in oceanography. Edited by T. R. Gibbs. pp.16–21 A.C.S. Washington, D. C. 1975

Eisler, R. G., G. E. Zaroogian and R. J. Hennekey: Cadmium uptake by marine organisms. J. Fish. Res. Bd Can. *29*, 1367–1369 (1972)

Elderfield, H., L. Thornton and J. S. Webb: Heavy metals and oyster culture in Wales. Mar. Pollut. Bull. *2*, 44–47 (1971)

Frazier, J. M.: The dynamics of metals in the American oyster, *Crassostrea virginica*. 1. Seasonal effects. Chesapeake Sci. *16*, 162–171 (1975)

Galtsoff, P.: The American oyster, *Crassostrea virginica* (Gmelin). Fishery Bull. Fish. Wildl. Serv. U.S. *64*, 1–480 (1964)

George, S. G. and T. L. Coombs: The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. Mar. Biol. *39*, 261–268 (1977)

Greig, R. A., B. A. Nelson and D. A. Nelson: Trace metal content in the American oyster. Marine Poll. Bull. *6*(5), 72–73 (1975)

Greig, R. A. and D. R. Wenzloff: Metal accumulation and depuration by the American oyster *Crassostrea virginica*. Bull. Environ. Contam. Toxicol. *20*, 499–504 (1978)

Haug, A., S. Melsom and S. Omang: Estimation of heavy metal pollution in two Norwegian fjord areas by analysis of the brown alga *Ascophylum nodosum*. Envir. Pollut. *7*, 173–193 (1974)

Kerfoot, W. B. and S. A. Jacobs: Cadmium accrual in combined wastewater treatment – aquaculture system. Environ. Sci. and Tech. *10*(7), 662–667 (1976)

Kopfler, F. C. and J. Mayer: Studies on trace metals in shellfish. Proc. Gulf and South Atlantic Shellfish Sanitation Res. Conf. 67–80 (1969)

Kurland, L. T., S. N. Faro and H. Siedler: Minamata disease: the outbreak of neurological disorders in Minamata, Japan and its relationship to the ingestion of seafood contaminated by mercury. Wld. Neurol. *1*(5), 370–391 (1960)

MacKay, N. J., R. J. Williams, J. L. Kacprzac, M. N. Kazacos, A. J. Collins and E. N. Auty: Heavy metals in cultivated oysters (*Crassostrea virginica* = *Saccostrea cucullata*) from the estuaries of New South Wales. Aust. J. Mar. Freshwat. Res. *26*, 31–46 (1975)

Noel – Lambot, F.: Distribution of cadmium, zinc and copper in the mussel *Mytilus edulis*. Existence of cadmium-binding proteins similar to metallothioneins. Experientia *32*, 324–326 (1976)

Phillips, D. J. H.: The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. 1. Effects of environmental variables on uptake of metals. Mar. Biol. *38*, 59–69 (1976)

Pringle, B. H., D. H. Hissong, E. L. Katz and S. T. Mulawka: Trace metal accumulation by estuarine molluscs. J. Sanit. Engng. Div. Am. Soc. Civ. Engrs. *94*, 455–475 (1968)

Ratkowsky, D. A., S. J. Thrower, I. J. Eustace and J. Olley: A numerical study of the concentration of some heavy metals in Tasmanian oysters. J. Fish. Res. Bd Can. *31*, 1165–1171 (1974)

Read, K. H.: Respiration of the bivalve molluscs *Mytilus edulis* L. and *Brachiodontes demissus plicatulus* Lamarck as a function of size and temperature. Comp. Biochem. Physiol. *7*, 89–101 (1962)

Romeril, M. G.: The uptake and distribution of ^{65}Zn in oysters. Mar. Biol. *9*, 347–354 (1971)

Roosenburg, W. H.: Greening and copper accumulation in the American oyster, *Crassostrea virginica*, in the vicinity of a stream electric generating station. Chesapeake Sci. *10*, 241–252 (1969)

Schulz – Baldes, M.: Lead uptake from seawater and food, and lead loss in the common mussel *Mytilus edulis*. Mar. Biol. *25*, 177–193 (1974)

Shuster, C. and B. H. Pringle: Trace metal accumulation by the American oyster, *Crassostrea virginica*. Proc. Nat. Shellfish Assoc. *59*, 93–103 (1969)

Watling, H. R. and R. J. Watling: Trace metals in oysters from the Knysna estuary. Mar. Pollut. Bull. *7*, 45–48 (1976)

Windom, H. L. and R. G. Smith: Distribution of iron, magnesium, copper, zinc and silver in oysters along the Georgia coast. J. Fish. Res. Bd Can. *29*, 450–452 (1972)

Zaroogian, G. E.: Studies on the depuration of cadmium and copper by the American oyster *Crassostrea virginica*. Bull. Environ. Contam. Toxicol. *23*, 117–122 (1979)

Zaroogian, G. E., G. Morrison and J. F. Heltshe: *Crassostrea virginica* as an indicator of lead pollution. Mar. Biol. *52*, 189–196 (1979)

Date of final manuscript acceptance: May 29, 1980.
Communicated by I. Morris, West Boothbay Harbor